

CHOO et al. - Appln. No. 08/793,408

Insert the attached paper copy of the Sequence Listing in lieu of pages 52-58 of the specification (i.e., the Sequence Listing submitted on February 20, 1997), and number pages accordingly.

IN THE CLAIMS:

Please amend the claims as follows.

C1
~~1~~
1. (Amended) A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display [on a viral particle], the zinc finger polypeptide comprising at least [three zinc fingers, with] one zinc finger having partially randomised allocation of amino acids [being positioned between two or more zinc fingers having defined amino acid sequence], the partially randomised zinc finger having a random allocation of amino acids at positions -1, +2, +3 and +6 and at least one of positions +1, +5 or +8, position +1 being the first amino acid in the α -helix of the zinc finger.

Claim 3, line 1, replace "claim 2" with --claim 1--.

~~4~~
C2
4. (Amended) A library according to claim 1 [in a form suitable for cloning] as a fusion with a DNA sequence encoding the minor coat protein of bacteriophage fd.

11 5. (Amended) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising of steps ^{of the} ~~of the~~

D screening against at least a portion of the target DNA sequence, ~~a plurality of~~ zinc finger polypeptides having ^a partially randomised zinc finger [positioned between two or more zinc fingers having defined amino acid sequence], the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the ~~plurality~~ ~~of~~ zinc finger polypeptides being encoded by a library in accordance with claim 1; and

D selecting those nucleic acid sequences encoding randomised zinc fingers which bind to the target DNA sequence.

5 5. (Amended) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

14 comparing the binding to one or more DNA triplets of each of a plurality of zinc finger polypeptides having a partially randomised zinc finger [positioned between two or more zinc fingers having defined amino acid sequence], the zinc finger

polypeptides being encoded by a library in accordance with claim 1; and

selecting those nucleic acid sequences encoding randomised zinc fingers [exhibiting preferred binding characteristics] which bind to the target DNA sequence.

35 6. (Amended) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

screening against at least a portion of the target DNA sequence, a plurality of zinc finger polypeptides having a partially randomised zinc finger [positioned between two or more zinc fingers having defined amino acid sequence], the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the plurality of zinc finger polypeptides being encoded by a library in accordance with claim 1;

comparing the binding to one or more DNA triplets of each of [a] said plurality of zinc finger polypeptides having a partially randomised zinc finger positioned between two or more zinc fingers having defined amino acid sequence; and

5
7 selecting those nucleic acid sequences encoding randomised zinc fingers [exhibiting preferred binding characteristics] which bind to the target DNA sequence.

7. (Amended) A method of designing a zinc finger polypeptide for binding to a particular target DNA sequence, the method comprising the steps of:

D screening against at least a portion of the target DNA sequence, ~~a plurality of~~ zinc finger polypeptides having a partially randomised zinc finger [positioned between two or more zinc fingers having defined amino acid sequence], the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the ~~plurality~~ ~~of~~ zinc finger polypeptides being encoded by a library in accordance with claim 1;

D comparing the binding to one or more DNA triplets of each of [a] said ~~plurality of~~ zinc finger polypeptides having a partially randomised zinc finger [positioned between two or more zinc fingers having defined amino acid sequence];

selecting certain of the screened randomised zinc fingers for analysis of [preferred] binding characteristics;

C5 and combining those sequences encoding desired zinc fingers to form a sequence encoding a single zinc finger polypeptide [having the desired binding specificity].

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D 10. (Amended) A method of designing a zinc finger polypeptide for binding to a particular ~~DNA~~ target sequence, wherein ~~a plurality of~~ sequences encoding individual zinc fingers selected by the method of claim 11 are randomly combined in the appropriate order to encode ~~a plurality of~~ zinc finger polypeptides, the zinc finger polypeptides being screened against the target sequence, that combination of zinc finger sequences encoding a zinc finger polypeptide [having optimal binding characteristics] which binds to the target DNA sequence ~~being selected for use~~.
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D
D
36
D
D

19 11. (Amended) A DNA library according to claim 1, consisting of 64 sequences, each sequence comprising a different one of the 64 possible permutations of a DNA triplet, the library being arranged in twelve sublibraries, wherein for any one sub-library one base in the triplet is defined and the other two bases are randomised[, the sequences being in a form suitable for use in the selection method of claim 8].

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21 ~~12~~. (Amended) A library according to claim ~~11~~, wherein the sequences are associated[, or are capable of being associated,] with separation means.

C6 22 ~~13~~. (Amended) A library according to claim ~~12~~, wherein the separation means is selected from the group consisting of microtitre plate, magnetic bead, non-magnetic bead, sedimentation particle, and affinity chromatography column [one of the following: microtitre plate; magnetic or non-magnetic beads or particles capable of sedimentation; and an affinity chromatography column].

23
J7 23 ~~15~~. (Amended) A kit for making a zinc finger polypeptide for binding to a nucleic acid sequence of interest, comprising: a library of DNA sequences according to claim 1 encoding zinc finger polypeptides, [of known binding characteristics] ~~in a form suitable for cloning~~ into a vector; ^{that accepts} a vector molecule ~~suitable for accepting~~ one or more sequences from the library; and instructions for use.

25 ~~14~~. (Amended) A kit according to claim ~~13~~, wherein the vector [is capable of directing] directs the expression of the cloned sequences as a single zinc finger polypeptide displayed on the surface of a viral particle.

Claim 20, line 1, replace "claim 18" with --claim 19--.

C9 15¹⁴ 23. A method according to claim 22, further comprising the step of separating the zinc finger polypeptide and the sequence of interest specifically bound thereto, [(and nucleic acid sequences specifically bound thereto)] from the rest of the sample.

Please cancel claims 18, 23-31 and 37-42 without prejudice and add the following new claims.

C10 8¹³ 43. A method for producing a zinc finger polypeptide for binding to a particular target DNA sequence, comprising the steps of:

screening against at least a portion of the target DNA sequence, ~~a plurality of~~ zinc finger polypeptides having a partially randomised zinc finger, the portion of the target DNA sequence being sufficient to allow binding of some of the zinc finger polypeptides, the ~~plurality of~~ zinc finger polypeptides being coded by a library in accordance with claim 1;

selecting those nucleic acid sequences encoding randomised zinc fingers which bind to the target DNA sequence; and

expressing the selected nucleic acid sequences to produce zinc finger polypeptides which bind to the target DNA sequence.

9. ~~44~~. A library according to claim 1, wherein the zinc finger polypeptide is displayed on a viral particle.

10. ~~45~~. A library according to claim 1, wherein the partially randomised zinc finger is positioned between two or more zinc fingers.

C10 30 ~~46~~. A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display, the zinc finger polypeptide comprising at least one zinc finger having partially randomised allocation of amino acids, the partially randomised zinc finger having a random allocation of amino acids at positions -1, +1, +2, +3 and +6, position +1 being the first amino acid in the α -helix of the zinc finger.

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31 ~~47~~. A library according to claim ~~46~~, wherein the partially randomised zinc finger further has a random allocation of amino acids at position +5.

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32 ~~48~~. A library according to claim ~~47~~, wherein the zinc finger polypeptide is displayed on a viral particle.

33 31
~~45~~. A library according to claim ~~47~~, wherein the partially randomised zinc finger is positioned between two or more zinc fingers.

34 30
~~50~~. A library according to claim ~~46~~, wherein the partially randomised zinc finger further has a random allocation of amino acids at position +8.

C10 35 34
~~51~~. A library according to claim ~~50~~, wherein the zinc finger polypeptide is displayed on a viral particle.

36 34
~~52~~. A library according to claim ~~50~~, wherein the partially randomised zinc finger is positioned between two or more zinc fingers.

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~~53~~. A library of DNA sequences, each sequence encoding a zinc finger polypeptide for display, the zinc finger polypeptide comprising at least one zinc finger having partially randomised allocation of amino acids, the partially randomised zinc finger having a random allocation of amino acids at positions -1, +2, +3, +5 and +6, position +1 being the first amino acid in the α -helix of the zinc finger.